Insect Pathogens and Insect Parasitic Nematodes
IOBC Bulletin Vol. 21 (4) 1998
pp.229 - 233

Potential impact of larval behavior on the use of transgenic crops expressing *Bacillus thuringiensis* toxins

Stuart R. Reitz, John T. Trumble
Department of Entomology, University of California, Riverside, California 92521, USA

Introduction

Over the last two decades *Bacillus thuringiensis* (Bt) based Integrated Pest Management programs in vegetable crops, including tomatoes and celery, have successfully controlled many insect pests in an environmentally safe manner while maximizing economic return for the *19, 20*. With the development of new molecular techniques, it is now possible to insert genes coding for Bt protoxins into plants. While the use of toxin-expressing transgenic plants has great potential for pest regulation, we believe that much information is still needed to efficiently utilize this technology.

Several authors have indicated the urgent need of basic information on the effect of Bt toxins on larval behavioral ecology in order to improve decisions on gene *6, 11*. However, to date we have behavioral information on only a handful of insect pests.

Here we discuss the possible impact of our results concerning effects of Bt toxins on larval behavioral ecology of *Spodoptera exigua* (Hübner), on the use of transgenic plants expressing Bt toxins for pest regulation. For this, we identified three major areas of impact, these are: 1) lack of basic information on behavioral ecology; 2) possible effects of larval behavioral ecology on pesticide resistance; and 3) possible effects of larval behavioral ecology on the integration of this technology within an IPM framework. Furthermore, we discuss potential problems with consumer acceptance of this technology.

• Non-random dispersal by this insect (Fig. 1) accentuates the possibility that the larvae can develop to older and more Bt tolerant stages on preferred hosts such as weeds *(Chenopodium murale* L.) and then move to the transgenic crops. Older larvae of several species have been shown to be less susceptible to Bt toxins than younger *1, 22, 23*. Therefore, mortality levels of older less susceptible larvae that developed on weeds before moving onto transgenic plants may be lower than expected from non-choice tests with neonates.

• Avoidance of Bt toxin (Fig. 2) by *S. exigua* larvae might have a negative impact on the effectiveness of seed mixtures. The larvae could prefer the susceptible cultivar and develop to a late instar and become more tolerant to the Bt toxin in the transgenic plants. This problem is accentuated by avoidance of toxins by CryIC-resistant *S. exigua* larvae (Fig. 3).

• The characteristics of the cultivar into which the Bt gene is inserted can interact with the toxin in their effect on larval behavior *16*. The interaction between the behavioral effect on *S. exigua* larvae of CryIC toxins and host plant characteristics (Fig. 4) indicates the need to obtain behavioral ecology information for each specific system.

Possible effects of larval behavioral ecology on pesticide resistance

• Possible avoidance of transgenic plants by susceptible *S. exigua* larvae (Figs. 2 & 4) might delay the development of resistance when using seed mixtures or tissue specific expression *4, 11, 17*.

• *S. exigua* larvae selected for resistance to CryIC showed cross resistance to CryIA(b), CryIE/CryIC fusion protein, CryIH, and CryIIA *13*. Therefore, transgenic toxin mixtures, such as those used by van der Salm et al. *(21)* where they fused CryIC-CryIA(b) genes, may not delay development of resistance in this insect.

• Possible avoidance of transgenic plants by resistant *S. exigua* larvae (Fig. 3) may further reduce the fitness differential between susceptible and resistant larvae, thereby reducing the development of resistance.

• Genes commercially available as sprays (e.g., CryIC, CryIA(b)) and to which pests have been shown to develop cross resistance should be avoided for the development of transgenic plants *9, 18, 24*.

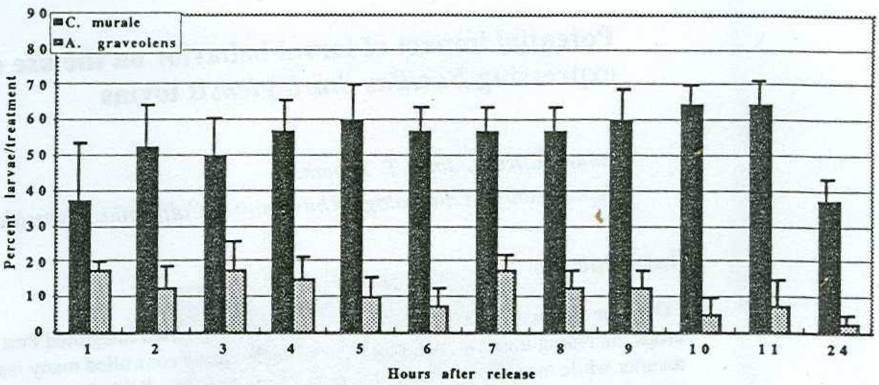
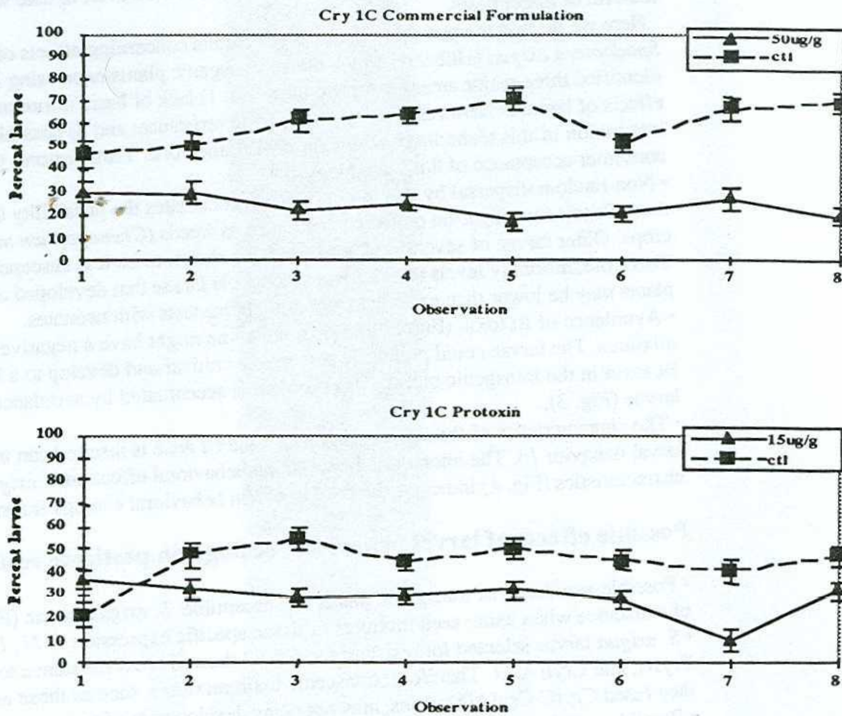


Fig. 1. In greenhouse choice tests between *Chenopodium murale* L. (Nettleleaf goose foot) and *Apium graveolens* L. (celery) plants, third instar *S. exigua* showed preference for *C murale* over *A. graveolens* ($P<0.05$)2.



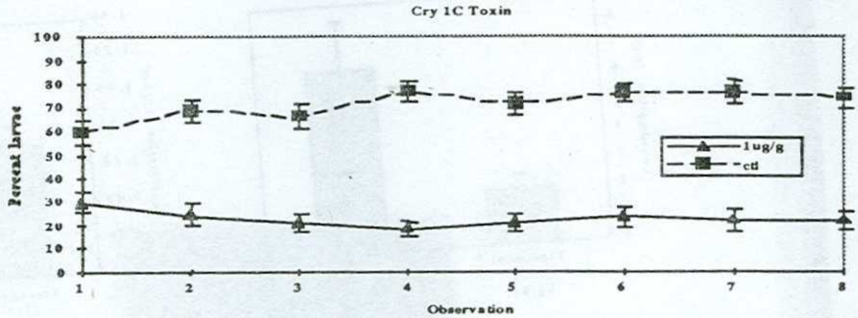


Fig. 2. In choice tests between CryIC-diet and control diet, first and third instar (data not shown) *S. exigua* showed avoidance of all CryIC forms tested, commercial formulation, CryIC protoxin, and trypsinized CryIC toxin (P < 0.005) *3*. Observations made at 12 hour intervals.

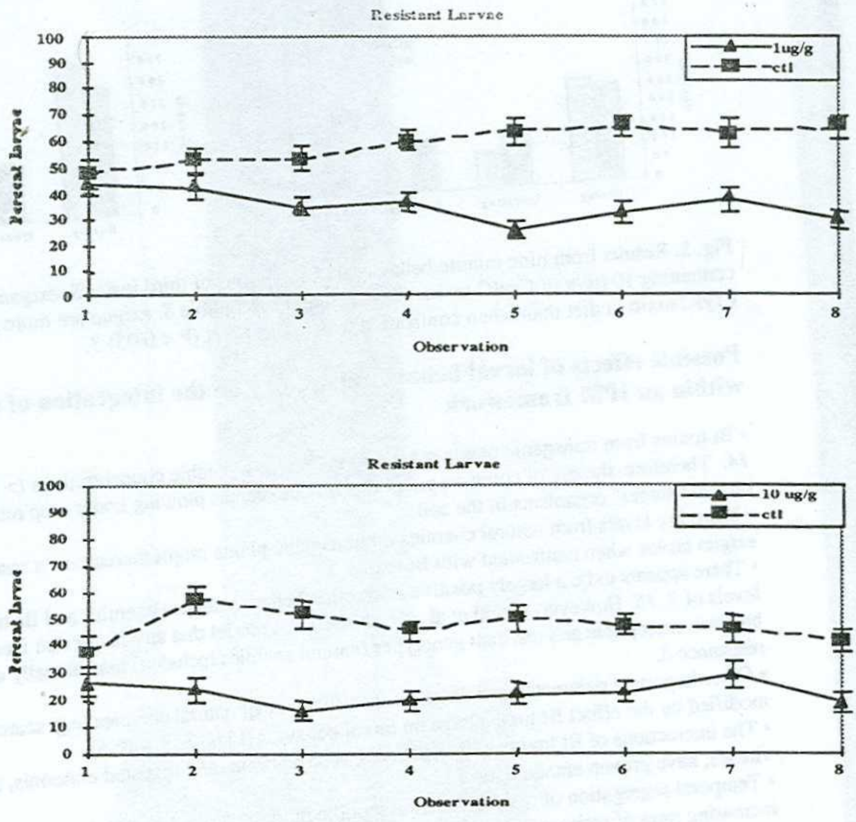


Fig. 3. In choice tests between trypsinized CryIC diet and control diet, neonate and third instar CryIC *resistant S. exigua* showed avoidance of trypsinized CryIC diet (P < 0.0001) *3*. Observations made at 12 hour intervals.

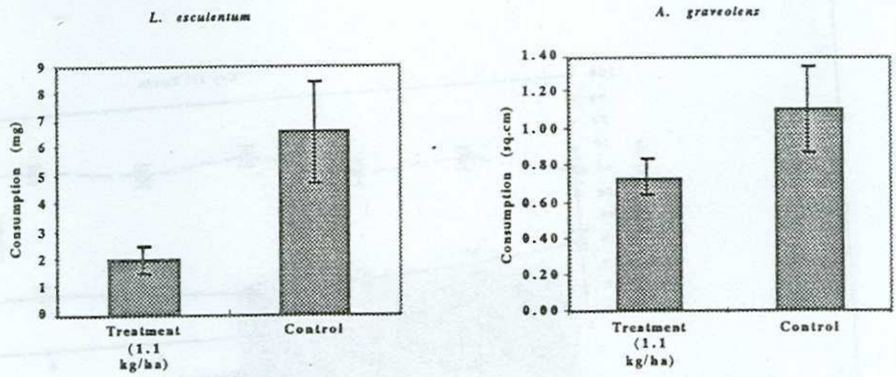


Fig. 4. In choice tests, third instar *S. exigua* showed reduced consumption of *Lycopersicon esculentum* Miller (tomato) leaves sprayed with a CryIC commercial formulation at the recommended rate of 1.1 kg [AI]/ha ($P < 0.05$). However, in choice tests between Bt sprayed and control *A. graveolens* leaves, third instar *S. exigua* showed no significant differences in leaf area consumption ($P > 0.05$) (Berdegué and Trumble, unpublished).

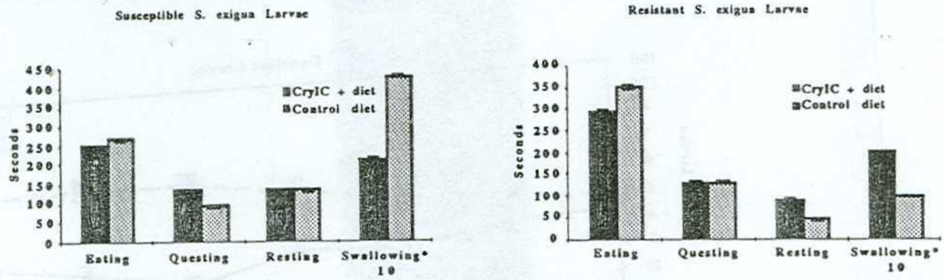


Fig. 5. Results from nine minute behavioral observations of third instar *S. exigua* when confronted with diet containing 10 µg/g of CryIC toxin. Resistant and susceptible *S. exigua* are more active when confronted with CryIC toxin in diet than when confronted with control diet ($P < 0.05$) *3*.

Possible effects of larval behavioral ecology on the integration of this technology within an IPM framework

- Bt toxins from transgenic plants remain in the soil at traceable concentrations (> 0.5 ng/g soil) for up to 30 days *14*. Therefore, the use of common cultural practices such as plowing under crop residues might have negative effects on "non-target" organisms in the soil.
- Mortality levels from natural enemies on transgenic plants might increase as a result of increased activity of *S. exigua* larvae when confronted with Bt toxins.
- There appears to be a largely positive interaction between natural enemies and Bt-transgenic plants expressing low levels of *7, 15*. However, Gould et al. *(5)* showed in a model that any factor that increases the fitness differential between susceptible and resistant genotypes (natural enemies included) may actually accelerate the development of resistance *8*.
- Other important parameters which affect the efficiency of natural enemies (e.g. searching efficiency) may be modified by the effect Bt toxins have on larval behavior (Figs. 2, 3, 4 & 5).
- The interactions of Bt toxins with repellents, such as neem, and ingested materials, such as abamectins or insect viruses, have proven antagonistic *12*.
- Temporal segregation of resistant and susceptible individuals could result in assortative mating *10*, thereby increasing rates of resistance development to transgenic plants.

Problems with consumer acceptance

• Avoidance of Bt toxins by *S. exigua* (Figs. 2, 3 & 4) might increase the efficiency of tissue-specific expression of Bt toxins where the toxin is expressed only in harvestable plant parts *4*. Conversely, higher expression of the toxin in leaves relative to the fruit (e.g., tomatoes) could drive pests to feed preferentially on the fruit.
• The recent introduction of genetically engineered tomatoes has shown that the public can develop negative perceptions of such technologies (even for something as minimal as a 5 gene deletion). Lack of laws requiring public notification that fruit are from genetically engineered plants has reduced this problem. However, the potential for misunderstandings and public distress following the incorporation of "toxins" (regardless that Bt toxin proteins are safe for mammalian consumption is of a substantially greater magnitude than for any previously genetically-engineered plant.
• Recent negative reports on transgenic crops in the mass media (e.g., National Public Radio; March 18, 1996) on accounts of allergic reactions by consumers to "nutritionally based" transgenic soybeans indicate the potential of consumer adversity to these crops.

References

1 Ali, A. & S.Y. Young. 1996. J. Entomol. Sci. 31:1-8.
2 Berdegué, M. & J.T. Trumble. 1996. Entomol. Exp. Appl. 73:253-262.
3 Berdegué, M.; J.T. Trumble & W.J. Moar. 1996. Entomol. Exp. Appl. 80: 389-401.
4 Gould, F. 1988. Trends in Ecology and Evolution 3: 515-518.
5 Gould, F.; G.G. Kennedy & M.T. Johnson. 1991. Entomol. Exp. Appl. 58: 1-14.
6 Hokkanen, H.M.T. & C.H. Wearing. 1994. Biocontrol Sci. Tech. 4: 399-403.
7 Johnson, M.T. & F. Gould. 1992. Environ. Entomol. 21: 586-597.
8 Johnson, M T; F. Gould & G.G. Kennedy. 1997. Entomol. Exp. Appl. 82: 219-230.
9 Kennedy, G.G. & M.E. Whalon. 1995. J. Econ. Entomol. 88: 454-460.
10 Klepetka, B & F. Gould. 1996. Environ. Entomol. 25: 993-1001.
11 Mallet, J. & P. Porter. 1992. Proc. Royal Society London- Section B 250:165-169.
12 Moar, W. J. & J.T. Trumble. 1987. J. Econ. Entomol. 80:588-592.
13 Moar, W. J.; M. Pusztai-Carey; H. Van Faassen; D. Bosch; R. Frutos; C. Rang; K. Luo & M. J. Adang. 1995. Appl. Environ. Microbiol. 61: 2086-2092.
14 Palm, C.J.; K. Donegan; D. Harris & R.J. Seidler. 1994. Molecular Ecology 3:145-151.
15 Riggin-Bucci, T M & F. Gould. 1997. J. Econ. Entomol. 90: 242-251.
16 Robison, D.J.; McCown, B.H. & K.F. Raffa. 1994. Environ. Entomol. 23:1030-1041.
17 Tabashnik, B.E. 1994. Proc. Royal Society London -Section B 155: 7-12.
18 Tabashnik, B. E.; Y. B. Liu; N. Finson, N.; L. Masson & D.G. Heckel. 1997. Proc. Nat. Acad. Sci. United States. 94: 1640-1644.
19 Trumble, J.T.; W.G. Carson & K.K. White. 1994. J. Econ. Entomol. 87:1463-1469.
20 Trumble, J T; W.G. Carson & G.S. Kund. 1997. J. Econ. Entomol. 90: 139-146.
21 van der Salm, T.; D. Bosch; G. Honée; L. Feng; E. Munsterman; P. Bakker; W.J. Stiekema & B. Visser. 1994. Plant Molecular Biology 26: 51-59.
22 Whalon, M.E. & Wierenga, J.M. 1994 Biocontrol Sci. Tech. 4: 555-561.
23 Wierenga, J M; D. L. Norris & M. E. Whalon. 1996. J. Econ. Entomol. 89: 1047-1052.
24 Wigley, P. J.; C. N. Chilcott & A. H. Broadwell. 1994. Biocontrol Sci. Tech. 4: 527-534.